ULTRAPURE HYALURONIC ACID AND THE USE THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This is a continuation, of application Ser. No. 623,333, filed Oct. 17, 1975 now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to an ultrapure, high molecular weight hyaluronic acid, generally, but not necessarily in the form of the sodium salt (hereinafter referred to as "HUA"), obtained from animal connective tissue such as rooster combs, human umbilical cords, or from bacteria culture which is suitable for use as a biologically active therapeutic injection, implant or infusion because of its non-inflammatory properties when so used. As used herein, in connection with the HUA of this invention, non-inflammatory signifies the absence of significant cellular infiltration of the vitreous and anterior chamber, absence of significant flare in the aqueous humor, absence of significant pathological changes to the cornea, lens, iris, retina and choroid of the owl monkey eye when an HUA preparation is tested in accordance with the modified owl monkey test described below.

The invention also relates to processes for obtaining such product and the use thereof.

2. The Prior Art

HUA is a naturally occurring high viscosity glycosaminoglycan having alternating β 1-3 glucuronidic and β 1-4 glucosaminidic bonds. The molecular weight of this material is generally within the range of 50,000 to 8,000,000 (although there are reports of HUA having molecular weights as high as 13,000,000) depending on the source, method of isolation and method of determination. It is found in animal tissue, e.g., in umbilical cord, vitreous humor, synovial fluid, rooster combs, pathologic joints, group A and C hemolytic streptococci and in skin.

The isolation and characterization of HUA is described in Meyer et al, J. Biol. Chem. 107, 629 (1934); J. Biol. Chem. 114, 689 (1936); Balazs, Fed. Proc. 17, 1086 (1958); Laurent et al; Biochim. Biophys. Acta 42, 476 (1960). The structure of HUA was elucidated by Weissman et al, J. Am. Chem. Soc. 76, 1753 (1954) and Meyer, Fed. Proc. 17, 1075 (1958).

It has long been an object of medical researchers to obtain an HUA preparation from animal tissues which could be used as a substitute or partial substitute for the naturally occurring HUA in vitreous humor and/or synovial fluid. Moreover, a suitable HUA preparation has long been sought for other medical applications wherein there is a depletion of the naturally occurring fluids containing HUA and consequently, the need for a replacement for such fluids.

There is, therefore, a large body of literature describing so-called purified HUA and various techniques for purifying naturally occurring HUA. There are many high molecular weight purified HUA preparations which are described in the literature; however, none of these preparations is suitable for the above-described uses because there is some unidentified impurity present in all of them which causes severe inflammation when the preparation is injected into a mammalian body and particularly, the eyes, for the purpose of providing a substitute for a natural material such as vitreous humor. The preparation according to the invention does not contain this impurity because when it is used as a substitute for the naturally occurring material, no such inflammation is observed. To date, I have been unable to identify this impurity, although I am able to effectively demonstrate its presence in previously known preparations and its absence from the present preparation as will appear below.

There will now follow a description of the most relevant prior art of which I am aware.

In two publications by Balazs and Sweeney, there is described certain work done by them on the therapeutic use of so-called "reconstituted vitreous" which is a mixture of human collagen and human HUA. This reconstituted vitreous contains only a relatively low concentration of HUA, i.e., 0.1-0.3%, and thus, it was not of critical importance that the HUA be extremely pure. This work is described in (1) New and Controversial Aspects of Retinal Detachment, Chapter 36: "The Injection of Hyaluronic Acid and Reconstituted Vitreous into the Vitreous Cavity,**38** pages 371-376. Hocher Medical Division, Harper & Row, 1968; and (2) The Replacement of the Vitreous Body in the Monkey by Reconstituted Vitreous and by Hyaluronic Acid, Mod. Probl. Ophthal., Vol. 4, pp. 230-232, 1966. These papers describe the use of an HUA preparation which was thought at the time to be acceptable because "Postoperative reaction in the anterior chamber (of the monkey eye) showed a slight flare and a little fibrinous precipitation which disappeared in the majority of cases after several days" Mod. Probl. Ophthal., page 230. It is to be noted of course, that fibrous precipitate in the anterior chamber is the result of excessive inflammatory reaction and consists of precipitated fibrin and agglomerated inflammatory cells. It has since been learned that the HUA preparation described in these references contained far too much of the unidentified inflammatory fraction of HUA. The preparatory method decribed in these publications will not lead to the obtention of an HUA free of inflammatory fractions.

A series of publications by Constable and Swann:

(1) Biological Vitreous Substitutes — Inflammatory Response in Normal and Altered Animal Eyes, Arch. Ophthal., 88, Nov. 1972, 544-548;

(2) Vitreous Structure II Role of Hyaluronate, Invest. Ophthal., 11, No. 3, pp. 164-168, 1972; and

(3) Vitreous Substitution, Retina Congress, Eds. Pruit and Regen, Appleton, Century and Croft, 1974, pps. 709-713,

describes the preparation and use (as a vitreous substitute) of a purified HUA. This preparation, which is somewhat similar to mine is, nevertheless not suitable as a vitreous substitute because it too is characterized by the presence of some unidentified inflammation causing impurity. Thus, on page 545 of the Arch. Ophthal. article, the authors state that "Hyaluronic acid in normal eyes caused a maximal 2+ or 3+ inflammatory response at two days (FIG. 1) which subsided clinically over seven to ten days. At two days the mean vitreous cell count was 1,194/cu mm and the protein concentration five times normal. Both cell counts and protein concentration rapidly fell to normal over three weeks." Moreover, on page 711 and 712 of Vitreous Substitution they state that "In normal owl monkey eyes [HUA] causes a mild acute inflammatory re-